CHARGE NUMBER: 6902

PROGRAM TITLE: BIOLOGICAL SPECIAL INVESTIGATIONS

PERIOD COVERED: AUGUST 1 - 31, 1984

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DATE OF REPORT: SEPTEMBER 7, 1984

I. TSNA IN FILLER: MODEL STUDIES (1,2,3)

Further investigations were conducted this month on the problem of TSNA formation during air curing of tobacco filler. As described in the July report, a modified homogenized leaf curing system was used as a model.

An aqueous slurry was prepared by grinding green, field-grown bright tobacco (4) in a blender. Particulate matter was filtered on a celite bed, and the filtrate was divided into four portions, each representing 4.5g dry weight. Nicotine citrate (50 mg of nicotine) was added to each portion. Sodium nitrate (15 mg NO_3^--N) was added to two portions, and potassium nitrite (15 mg NO_2^--N) to the remaining two portions. One of each of the nitrate and nitrite-enriched portions was sterilized by 0.2μ filtration, after which all four samples were statically incubated at 37°C for 24 hr followed by 20°C for 4 days. No substantial changes in nicotine content were observed. In the samples containing added nitrate, the presence of bacteria caused nitrate to be reduced to nitrite, and fairly high levels of TSNA were found. The sterile samples containing added nitrate did not undergo any changes in nitrate or nitrite, and only insignificant levels of TSNA were found. In the samples where nitrite was added, high levels of TSNA were found, regardless of whether bacteria were present, and nitrate/nitrite ratios did not change. It was concluded that the major role of bacteria is the reduction of nitrate to nitrite, and that the formation of TSNA occurs via chemical nitrosation of either the nicotine or secondary amine minor alkaloids.

The relative yields of TSNA from nicotine, nornicotine, anatabine and myosmine were determined by treatment of the alkaloids with nitrite in pH 5.5 citrate-phosphate buffer for 23 hours at 37.5°C. The results are shown below:

	% Yield			
	NNN	NNK	NNA	NAT
nicotine	0.007	0.017	0.16	0.019
nornicotine (5)	1.7	ND	ND	ND
anatabine	ND	ND	ND	8.4
myosmine (5)	ND	ND	ND	ND

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NNA and NNK are ring-opened compounds derived from nicotine. NNA is not isolated in the tobacco matrix, but has been observed in low temperature reactions previously (6). Since the yield of NNN from nicotine was only 1/250 that from nornicotine, the latter alkaloid is probably an important precursor of NNN in filler. The NNN/NNK ratio from nicotine was 1:2.4, as opposed to 15:1 from the bright tobacco model. This could also be due in part to the presence of nornicotine in tobacco, or to other factors such as pH, temperature, metal ions, etc. An attempt was made to find nornicotine in the base fraction from the initial filtrate from green burley, but none could be found (7). Further attempts will be made.

In another experiment using green field-grown bright tobacco, the effect of incomplete filtration or no filtration was determined. It was found that TSNA are produced if all the insoluble material is removed, but the presence of a green, colloidal suspension essentially eliminates TSNA production, and causes all nitrate to be removed without buildup of nitrite. When the filtrate was incubated at 37.5°C for 3 days, NNK could not be detected, but at 21°C/6 days the NNK was 2X the NNN. It was also shown that one organism, isolated in pure culture from the previous sample of bright tobacco, was capable of extensive reduction of nitrate to nitrite and production of TSNA when inoculated into a sterile filtrate and incubated at 37.5° for 3 days, while another organism isolated from the same sample was much less effective despite prolific growth in the model system.

Further experiments are being planned to study the effects of temperature, pH, inhibitors, etc., on TSNA production in these systems. The role and mechanism of action of the green collodial material will also be examined, as will the possible effect of metal ions.

II. REFERENCES

1. Warfield, A. N. Notebook 8063.
2. Yu, T. Notebook No. 7729.
3. Petri, D. L. Notebook No. 7701.
4. Obtained from D. Teng.
5. Obtained from H. Secor.
6. Hecht, S. S.; Chen, C. B.; Ornaf, R. M.; Jacobs, E.; Adams, J. D.; Hoffmann, D. Reaction of nicotine and sodium nitrite: formation of nitrosamines and fragmentation of the pyrrolidine ring. J. Org. Chem. 43, 72-76, 1978.
7. Kinser, R. Notebook No. 8137.

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Source: https://www.industrydocuments.ucsf.edu/docs/ttfm0000